Distribution, spore ontogeny and host reactions in *Acaulospora scrobiculata* (Diversisporales)

by

T. Muthukumar*\( ^{1} \), P.N. Damodaran, V. Rajeshkannan and K. Udaiyan

Microbiology Laboratory, Department of Botany, Bharathiar University,
Coimbatore - 641 046, Tamil Nadu, India

With 14 figures and 2 tables


Abstract: *Acaulospora scrobiculata* is distributed in different vegetation and soil types in Tamil Nadu, India, and proliferates in different plant species, but distribution and abundance of its spores were not influenced by soil properties. *A. scrobiculata* inhabits different plant species including cowpea (*Vigna unguiculata* (L.) Walp.), maize (*Zea mays* L.), sorghum (*Sorghum vulgare* L.), *Tephrosia* (*Tephrosia purpurea* Pers.) and sunnhemp (*Crotalaria juncea* L.). Spores were produced in a soil-sand mixture five to nine weeks after spore inoculation. Under sorghum spore production was maximal, while minimum sporulation was observed under sunnhemp. Sporogenesis starts with the differentiation of the sporogenous hyphae terminally along thin-walled coenocytic hyphae. The terminal part of the sporogenous hyphae swell as a sporiferous sacule primordium, which matures into a sporiferous sacule delimited by a septum in the lower channel of the stalk. The spore is produced laterally within the stalk of the sporiferous sacule. The cytoplasm in the sporiferous sacule stalk differentiates into regions of high and low density prior to spore primordium initiation. The spore differentiates laterally in the stalk and expands. After complete spore differentiation, a second septum is formed delimiting the terminal portion of the sporiferous sacule, which degenerates leaving a single scar. Direct germination of the sporiferous sacule without spore formation is reported for the first time in the Acaulosporaceae (Diversisporales).

Introduction

Gerdemann & Trappe (1974) recognized *Acaulospora* as one of the seven genera of arbuscular mycorrhizal (AM) fungi and later Morton & Benny (1990) included this genus in the family Acaulosporaceae. Spore differentiation in *Acaulospora* is known

---

*Corresponding author (e-mail: tmkum@yahoo.com)

to occur laterally along a sporogenous hypha of a fully inflated sporiferous saccule (Wu & Hung 1995, Stürmer & Morton 1999). At maturity, the sporiferous saccule collapses, leaving the empty shrunken saccule. Some members of this genus (e.g., *Acaulospora trappei* and *A. gerdemanni*) have been transferred into the genus *Archaeospora*, based primarily on DNA sequences, fatty acid profiles, immunological reactions against specific monoclonal antibodies and mycorrhizal morphology (Morton & Redeker 2001). *Trappe* (1977) originally described *Acaulospora scrobiculata* from Mexican, U.S. and Japanese collections of hypogeous fungi. Since then, there are several reports indicating the occurrence of this species world-wide (Musoko et al. 1994, Vestberg 1995, Guadarrama & Álvarez-Sánchez 1999, Muthukumar & Udaian 2000), and widely distributed in the different vegetation types in the Western Ghats region of southern India (Muthukumar & Udaian 2000/2002). Further *A. scrobiculata* is known to form typical vesicular-arbuscular mycorrhiza in host roots and to increase (Yano-Melo et al. 1999) or depress (Modjo et al. 1987) some aspects of plant growth in different plant species. However, no information exists on the influence of soil characters on the occurrence and abundance of *A. scrobiculata*. So the first aim of this investigation was to record the distribution of *A. scrobiculata* in different vegetation and soil types in Tamil Nadu and to assess the influence of soil characters on its distribution.

Because AM fungi are obligate symbionts, unable to be grown without a host, their proliferation depends on host plants (Struble & Skipper 1988, Simpson & Daft 1990, Liu & Wang 2003), substrate (Sreenivasas & Bagyaraj 1988), and environmental conditions (Brundrett et al. 1999). Influences of host species on sporulation of members of the Glomeraceae and Gigasporaceae are well known (Struble & Skipper 1988, Simpson & Daft 1990). However, no information is available on such influences for members of the Acaulosporaceae. So the second aim is to report the extent of sporulation of *A. scrobiculata* in relation to different host species.

Spore development in certain AM fungal genera (*Gigaspora* (Bentivenga & Morton 1995) and *Scutellospora* (Morton 1995)) or species [*Entrophospora kentiensis* (Wu & Hung 1995), honey-coloured sessile spores (Mosse 1970a, b, c)] has been studied in detail. Recently Dalpé & Declerck (2002) reported spore development in *A. rehmi* in root organ cultures. The third aim of this study was to trace spore development in *A. scrobiculata*.

**Materials and methods**

*DISTRIBUTION*: In March 2000, three adjacent soil cores (1.5 cm diam., 10 cm deep) were taken from five random points in each of six vegetation types (grassland, scrubland, forest, plantation, cultivated field and wasteland). The soils from three adjacent cores were pooled, mixed thoroughly, shade-dried and used for the determination of soil characteristics and AM fungal spore enumeration. So there were five composite soil samples for each vegetation type.

Soil pH was determined in a digital pH meter using a soil : water (1:2 v/v) suspension. Total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) in soil samples were determined by elemental analysis (Davis 1962, Jackson 1971).

AM fungal spores were extracted from 20 g rhizosphere air-dried soil using a modified wet-sieving technique (Muthukumar & Udaian 2000). Spores were recovered by filtering the sieved fraction
onto a filter paper. The filter paper was then spread over a glass plate and intact spores were counted according to morphologically distinct types and recorded as totals per sample under a dissecting microscope. Intact spores were mounted on slides and diagnostic features were recorded. Colour and dimensions of intact spores were assessed under the dissecting microscope using incident illumination. Spores were then mounted on microscope slides in polyvinyl-lactic acid-glycerol with or without Melzer's reagent and carefully crushed. The specimens were identified using published descriptions (Gerdemann & Trappe 1974, Trappe 1977, Nicolson & Schenck 1979, Schenck & Smith 1982, Walker 1982, Berch & Fortin 1983, Schenck et al. 1984, Koske 1985, Koske et al. 1986, Walker & Sanders 1986, Walker & Koske 1987, Almeida & Schenck 1990). Voucher specimens have been deposited in the Botany Department, Bharathiar University, Coimbatore, Tamil Nadu, India.

Influence of Host Species: To study the influence of different host species on spore production of *A. scrobiculata*, pot cultures were established in 12-cm diam. pots containing 850 g of autoclaved sand : alfisol soil (1:1, v/v) mixture with different host plants. Spores of *A. scrobiculata* isolated from the semi-arid soils (isolate BUCC 0158) (Muthukumar & Udayan 2002) were used and propagated in pot cultures with sorghum (*Sorghum vulgare* L.) as host plant. The host plants used were cowpea (*Vigna unguiculata* (L.) Walp.), maize (*Zea mays* L.), *Tephrira* (*Tephrira purpurea* Pers.), sorghum and sunhemp (*Crotalaria juncea* L.). An analysis of the soil mixture using standard methods (Jackson 1971, Davis 1962) indicated a pH of 7.4, 6.9 mg kg⁻¹ of total N, 0.6 mg kg⁻¹ of available P and 14.8 mg kg⁻¹ of exchangeable K. The soil mixture was autoclaved for 90 min. (121°C, 1.05 kg cm⁻²) on three separate days.

The soil was incubated for 15 days prior to use. The pot contents were wetted and a 1-cm diam. hole was drilled at the center to half way down each pot. Fifty spores of *A. scrobiculata* obtained by wet sieving and decanting technique (Muthukumar & Udayan 2000) were transferred to each hole. The hole was filled with sterile sand-soil mixture and two seeds of the appropriate host plant were sown on top of the filled hole. After emergence, the seedlings were thinned to one seedling per pot. The legumes were inoculated with 5 ml of their crushed nodule filtrate (passed through two layers of cheesecloth). The plants were watered as necessary and no fertilizer was applied. There were five pots for each host, resulting in 25 pots for all hosts. Two uninoculated pots were maintained for each host to check contamination.

To assess AM formation and sporulation in different hosts, 1-cm diam. and 10 cm deep (20 g) soil samples were taken at weekly intervals up to 12 weeks after sowing using a soil corer. The hole was filled with a sterile sand : soil mixture and the point was marked with a wooden toothpick to avoid future sampling of the same point. Samples were processed, spores counted and roots assessed to determine the extent of root length colonized. AM fungal spore numbers were counted and expressed as spore numbers per 10 g of soil. The roots were cleared, stained (Koske & Gemma 1989) and the extent of colonization was recorded according to the magnified intersection method (McGonigle et al. 1990).

Sporogenesis: To study sporogenesis, surface-sterilized seeds of sorghum were sown in a plastic tray (40 x 20 x 8 cm) containing the soil-based inoculum of *A. scrobiculata*. Fifteen days after germination, the seedlings were removed and the roots were washed free of adhering soil. The seedlings were transplanted at the rate of two seedlings per plastic pot (10 cm diam.) containing sterile sand : soil mixture (500 g). The soils were analysed for freshly formed spores 30 and 45 days after transplantation. There were five pots for each harvest resulting in a total of ten pots. At harvest the pot contents were carefully removed and soaked in a tub containing water. The tub was gently agitated for 15-20 min. to remove most of the soil adhering to the roots. The root systems were then transferred to a 35 mm sieve and rinsed in tap water until the root systems were free of soil and debris, leaving the extramatrical hyphae and spores attached to roots. The root systems were spread individually on 20-cm diam. Petri dishes in water and the extramatrical hyphae and spores attached to roots were removed using a fine forceps under a dissecting microscope (x 40) and mounted for observation. Different stages of spore development were mounted in polyvinyl alcohol-lactic acid-glycerol (PVLAG) (Omar et al. 1979), or PVLAG with Melzer's reagent (1:1, v/v) or 0.05% trypan blue in lactoglycerol. Sizes of spores and sporiferous sacules were measured with an ocular micrometer. Microscopic observations were made and photographed with a Nikon compound microscope equipped with phase-contrast optics.

Statistical Analysis: Data on soil variables, spore numbers and AM fungal colonization were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's Multiple Range Test.
(DMRT) when necessary. Pearson’s correlation analysis was used to assess the relationship between mycorrhizal and soil variables. Spore numbers were log-transformed (X + 1) and root colonization data were arcsine transformed prior to analysis.

Results and discussion

Habitat and distribution

Spores of *A. scrobiculata* were widely distributed in different vegetation and soil types (Table 1), which is in accordance with the widespread occurrence of this species in both disturbed and undisturbed ecosystems (Musoko et al. 1994, Jaiswal & Rodrigues 2001, Muthukumar & Udayan 2000, Vestberg 1995, Guadarrama & Alvarez-Sánchez 1999). One species of *Acaulospora*, seventeen species of *Glomus* and three species of *Scutellospora* were found to occur besides *A. scrobiculata*. The spore numbers of *A. scrobiculata* ranged between 2 to 15 spores in 100 g soil. However, the spore numbers of *A. scrobiculata* were not related (P > 0.05) to any soil factors examined (r values: pH = 0.528, N = 0.442, P = -0.120, K = 0.405). The lack of relationship between spore numbers and soil properties clearly indicates the capacity of *A. scrobiculata* to grow different soil types. Johnson (1993) also found no significant changes in *A. scrobiculata* spore populations in spite of eight years of continuous fertilization which significantly affected the populations of other AM fungal species in the AM fungal community. However, these observations do not exclude the possible occurrence of ecotypes within this species.

Influence of host species on sporulation

Root colonization by *A. scrobiculata* was characterized by typical arbuscules, vesicles and intraradical hyphae. No colonization was detected in uninoculated pots. Intraradical hyphae tend to be thicker (4-6 μm) and form more coils near the point of entry into a root. Morton & Redecker (2001) indicated that the varied staining intensities of AM fungal structures belonging to different genera could be used to distinguish them, however, the present study does not support this. Arbuscules often stain to varying intensities with trypan blue in different hosts. Gange et al. (1999) showed that the performance of a stain in detecting AM colonization is dependent on the plant species examined. Differences in the intensity of staining could be due to the differential penetration of the stain into roots of different plant species. In addition, the nature and location of the target material also has an effect, and it is possible that the depth at which arbuscules are located in the roots differs between plant species (Gange et al. 1999). These factors possibly could have contributed to the results observed in the present study. Vesicles ranged from spherical to oblong to irregular in shape and were often more abundant in the outer cortex or ground tissue. Root colonization by *A. scrobiculata* differed significantly with host species (F<sub>4,180</sub> = 64.18; P < 0.000) and plant growth (F<sub>7,160</sub> = 376.46; P < 0.000) (Fig. 1). The interaction (plant growth × host species) was also significant (F<sub>32,160</sub> = 4.93; P < 0.001).

Spore formation was observed in all inoculated host plants. This contrast with the observation of Liu & Wang (2003) who reported sporulation of *A. scrobiculata* in
Table 1. Occurrence of *Acaulospora scrobiculata* spores in different vegetation types, soil characteristics and accompanying arbuscular mycorrhizal (AM) fungal species.

<table>
<thead>
<tr>
<th>Vegetation type*</th>
<th><em>A. scrobiculata</em> spore numbers (in 100 g soil)†</th>
<th>Soil pH‡</th>
<th>Soil nutrients (mg kg⁻¹)§</th>
<th>Accompanying AM fungal species†‡§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland (Cymbopogon caesius)</td>
<td>10 de</td>
<td>6.1 ab</td>
<td>11.2 f</td>
<td>0.92 cd</td>
</tr>
<tr>
<td>Grassland (Heteropogon contortus)</td>
<td>12 de</td>
<td>6.3 bc</td>
<td>7.8 e</td>
<td>0.81 b</td>
</tr>
<tr>
<td>Grassland (Cymbopogon flexuosus)</td>
<td>10 de</td>
<td>7.6 g</td>
<td>7.3 c</td>
<td>0.81 b</td>
</tr>
<tr>
<td>Scrubland (Acacia spp.)</td>
<td>8 cd</td>
<td>7.8 gh</td>
<td>8.9 d</td>
<td>0.98 d</td>
</tr>
<tr>
<td>Forest</td>
<td>2 a</td>
<td>6.5 : cd</td>
<td>10.2 f</td>
<td>1.00 de</td>
</tr>
<tr>
<td>Forest</td>
<td>9 cd</td>
<td>6.9 f</td>
<td>9.8 e</td>
<td>0.96 d</td>
</tr>
<tr>
<td>Plantation (Acacia auriculiformis)</td>
<td>5 b</td>
<td>7.1 f</td>
<td>10.1 e</td>
<td>1.1 f</td>
</tr>
<tr>
<td>Plantation (Bambusa bambos)</td>
<td>6 bc</td>
<td>6.7 de</td>
<td>12.3 g</td>
<td>1.1 f</td>
</tr>
<tr>
<td>Plantation (Tectona grandis)</td>
<td>6 bc</td>
<td>6.8 e</td>
<td>6.2 b</td>
<td>0.86 bc</td>
</tr>
<tr>
<td>Cultivated field (Glycine max)</td>
<td>9 cd</td>
<td>7.9 h</td>
<td>26.2 j</td>
<td>1.21 g</td>
</tr>
<tr>
<td>Cultivated field (Sorghum vulgare)</td>
<td>15 e</td>
<td>7.6 g</td>
<td>15.1 h</td>
<td>0.53 a</td>
</tr>
<tr>
<td>Cultivated field (Gossypium hirsutum)</td>
<td>15 e</td>
<td>8.2 i</td>
<td>19.5 i</td>
<td>1.08 ef</td>
</tr>
<tr>
<td>Wasteland</td>
<td>3 a</td>
<td>5.9 e</td>
<td>4.3 a</td>
<td>0.53 a</td>
</tr>
</tbody>
</table>

F statistics (d.f. =12,52) | 18.15*** | 90.19*** | 811.69*** | 57.12*** | 779.25***

* Dominant or cultivated plant species in parenthesis.
† Means followed by same letter(s) do not differ significantly according to DMRT.
‡ *Glomus* spp. 1-6 are spores that do not fit into known descriptions.
*** Significant at P < 0.001.
Fig. 1. Percentage root length colonization by *Acaulospora scrobiculata* in five hosts during 12 weeks. Vertical bars indicate ± S.E.

soil trap cultures only in two of the four hosts tested. Spores were detected as early as five weeks after inoculation in sorghum and maize, and at nine weeks after inoculation in sunnhemp and *Tephrasia* (Fig. 2). Spore production of *A. scrobiculata* was significantly affected by host species (*F*$_{4,160}$ = 692.15; *P* < 0.001) and culture period (*F*$_{7,160}$ = 253.07; *P* < 0.001). The interaction (harvest × culture period) was also significant (*F*$_{28,160}$ = 18.45; *P* < 0.001). Of the five plant species evaluated *Tephrasia* and sunnhemp did not support abundant spore production of *A. scrobiculata*. Sorghum was the most effective for spore production followed by maize. This is in accordance with other reports (Simpson & Daft 1990, Brundrett et al. 1999, Liu & Wang 2003) where monocotyledonous plants tended to produce more spores per volume of soil than dicotyledonous species. The variation in different hosts might be due to differences in root type and morphology, biomass partitioning, nutrient requirement and exogenous hormonal level (Cuenca & Meneses 1996, Stutz & Morton 1996, Brundrett et al. 1999).

Spore production was significantly correlated (*P* < 0.01 to *P* < 0.001) to the extent of colonization in cowpea (*r* = 0.870; *P* < 0.002), maize (*r* = 0.945; *P* < 0.001), *Tephrasia* (*r* = 0.840; *P* < 0.005), sorghum (*r* = 0.950; *P* < 0.002) and sunnhemp (*r* = 0.799; *P* < 0.01). This conflicts with Gazey et al.'s (1992) view that root length colonized by a single AM fungal species is not necessarily correlated with the spore numbers produced on the same host. Smith & Read (1997) suggested that low nutrient concentrations are conducive to higher mycorrhization, so there may be a link between the extent of intraradical colonization and spore production. This was demonstrated for two species of *Acaulospora*, which required different critical lengths of intraradical colonization before sporulation commenced (Gazey et al. 1992). However, as root colonization by AM fungi and spore production are controlled by common host
Fig. 2. Spore production of *Acaulospora serobiculata* associated with different hosts. Vertical bars indicate ± S.E.

Factors like carbon allocation and low soil nutrient levels (Pearson & Schweigner 1993, Douds 1994), a correlation of these fungal variables is not surprising.

**Sporogenesis**

Hyphal morphology and mycorrhizal architecture of *A. serobiculata* were similar to other species in the family Acaulosporaceae (Wu & Hung 1995). Sporogenesis in *Acaulospora* is different from that of *Entrophosphora*. Spores of *Entrophosphora* are produced endogenously within the stalk of the sporiferous saccule, whereas in *Acaulospora* spore production is shifted laterally into the hyphal stalk. *A. serobiculata* typically produced spores singly in the soil. Various stages of spore ontogeny could be found in external mycelium. The extraradical hyphae are (2-3)(-3.5) μm wide, dichotomously branched, like those of other AM fungi. The early stage in sporulation of *A. serobiculata* is the differentiation of sporogenous hyphae. The density of the cytoplasm in an extraradical hypha increases 100-220 μm from the hyphal tip. The sporogenous hyphal apex inflates into a claviform shape, with the tip later swelling into a globose structure (Fig. 3). The sporiferous saccule increases in size and a septum always occurs in the lower part of the hyphal stalk of the saccule (Fig. 3). This contrasts with observations by Dalpé & Declerck (2002) for *A. rehmii*, where the sporogenous hyphae were not delimited by a septum. The cytoplasm within the saccule appears to flow towards the septum. A finger-like structure of cytoplasm, enclosed by a wall similar to that reported for *E. kentimensis*, extends towards the septum (Fig. 4) and delicate branches arise from the stalk (Fig. 5). The saccule is white to subhyaline, filled with dense cytoplasm. The saccule has a single hyaline layer with a smooth surface, 1.5-2.0 μm thick. The cytoplasm in the hyphal stalk is
later differentiated into regions of high and low density (Fig. 6). The sporiferous saccule also can germinate directly, producing germinal hyphae without spore formation. A kind of germination was noticed in <7% of the sporiferous saccules incubated in sterile wet sand (Fig. 7). This is the first report on the germination of the sporiferous saccule in the Acaulosporaceae.

The spore primordium differentiates laterally as a bud on this dense region of the sporiferous saccule stalk. Unlike A. rehnii, where the sporiferous saccule and the spore develop simultaneously, the sporiferous saccule in A. scrobiculata first reaches its maximum dimension of (119-182(-230) μm diam. before the spore primordium appears as a protuberance (Fig. 8). The spore develops laterally within a bud from the saccule neck and is attached to the sporiferous saccule neck by a short stalk (Figs 9, 11). Spores develop laterally at 40-80 μm distance from the saccule. The connection between saccule neck and spore is somewhat complex in that it initially consists of only the L1 layer of the spore (continuous with the wall of the saccule neck), followed by de novo synthesis of sublayers of the L2 (laminate) layer which form between saccule neck and spore. After degradation and sloughing of L1, these sublayers form a 2.8-4 μm high ridge around the occluded pore. The formation of this region proceeds like that observed during spore wall layer differentiation in Glomus species (Morton 1988).

As the spores enlarge and reach maturity, most of the cytoplasm is transferred into the saccule. A second septum is laid down in the saccule stalk nearer to the spore separating the saccule from the spore (Fig. 10). A similar type of wall formation has been reported in Entrophospora (Wu & Hung 1995). The pitted ornamentation on the spore is visible at this stage (Figs 9-11). As the spore matures, the saccule and hyphal stalk degenerate and leave a circular to ovoid, smooth scar, (7.5-10.7(-13.4) μm diam, on the surface (Fig. 12). Developmental studies of fungal isolates in INVAM indicate that mycorrhizal development and saccule formation are similar in Acaulospora and Entrophospora and those differences arise only as the spore bud begins to form laterally (Acaulospora) or within (Entrophospora) the neck of the fully expanded saccule (http://invam.caf.wvu.edu). Once the spore is positioned and has expanded to its full size, identical successional stages are seen in spore differentiation in both genera. The absence of any developmental differences between the two genera, except for the location of the spore in relation to the neck of the saccule, suggest a small positional shift in spore formation that had no effect on other events in spore subcellular differentiation.

At full differentiation, spores are sub-hyaline to pale yellow, globose, subglobose, occasionally irregular, (130-146(-235) X (105-146(-230)) μm; the spore wall consists

of a hyaline outer layer (L1), 0.8-1.2 μm thick, continuous with the wall of the neck of the parent sporiferous sacculum. The second (L2) and third (L3) layers are formed as the spore matures. The L2 layer thickens initially by formation of pale yellow to tan sublayers (or laminae) with ovoid concave depressions on the surface. These depressions are 0.5-2.3 μm across and 0.5-1.5 μm deep. Some merge together to form 4-10 μm long channels. Sublayers sometimes appear to split and consist of two groups of near-equal thickness, one including depressions and the other underlying the depressions, the composite thickness being (4.5-)6(-7.2) μm (Fig. 4c, e). The third layer (L3) adheres to L2 and separates only after a rather vigorous crushing but often appears as a lamina of L2.

Two flexible hyaline inner walls (gw1 and gw2) can be seen in all spores if they separate when the spore is broken (Fig. 13). The gw1 consists of two tightly adherent hyaline layers of near equal thickness, together measuring 1.0-1.3 μm, neither reacts in Melzer’s reagent. The gw2 also consists of two tightly adherent hyaline layers (gw211 and gw212). The gw211 is 0.5-1.2 μm thick, with beaded appearance, and gw212 is 1.2-1.5 μm thick and stains light purplish pink to reddish brown in Melzer’s reagent (Fig. 14). Trappe (1977) described the red reaction of the innermost spore wall layer in A. scrobiculata to be unique among the AM fungal species known till then. However, as observed in the present study, the reaction is amyloidal (purple), but because the yellow-stained outer wall acts as a filter, it might have given the impression that the innermost wall turns red (Walker et al. 2004).

Walker et al. (2004) discussed the use of the term “spore” in Acaulospora, as a result of a developmental sequence as shown in the present study. A rigid wall (exoospore) is first formed, followed by the development of a thin, flexible component (mesospore), and then an internal, completely separate structure (endospore) from which the germination shield forms (Spain 1992). However, the authors (Walker et al. 2004) retained the term “spore” for this complex structure which is considered to be a highly modified sporangiole, with the exospore corresponding to the sporangiophore, the mesospore to a sporangium, and the endospore to a sporangiospore. Researchers (Schenck et al. 1984) could have had considerable difficulty with diagnosis of Acaulospora species in part because of confusion concerning the beaded ornamentation on gw2 of A. spinosa and many other species.

Without this knowledge, the spores were often classified as belonging to a separate (usually undefined) species. It should be remembered that the granules on L1 of

Figs 8-14. Light and phase contrast micrographs of sporogenesis of Acaulospora scrobiculata. - 8. Primordial spore (sp) developing laterally on the stalk of a sporiferous sacculum (ss) (bar = 50 μm). - 9. Mature spore (sp) showing collapsed sacculum (bar = 50 μm). - 10. Second septum (s2) between the spore (sp) and the sporiferous sacculum (ss) (bar = 20 μm). - 11. Lateral attachment of spore with a short neck (arrow) on the hyphal stalk. Note the septum delimiting the sacculum terminus (s2) (bar = 20 μm). - 12. Spore surface showing pitted ornamentation and scar (arrow) (bar = 40 μm). - 13. Fractured spore exhibiting the wall layers (L1-3) and the two germinal walls (gw1 and gw2) (bar = 20 μm). - 14. Ruptured spore in Melzer’s reagent showing staining of the second germinal wall (arrow) (bar = 20 μm).
Table 2. Succession of spore differentiation stages in *Acaulospora scrobiculata* and *Acaulospora rehmi* (after Dalpé & Declerck 2002).

<table>
<thead>
<tr>
<th>Acaulospora scrobiculata</th>
<th>Acaulospora rehmi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporogenous hyphae differentiated terminally</td>
<td>Intercalary differentiation of the sporogenous hyphae</td>
</tr>
<tr>
<td>Sporogenous hyphae delimited by a septum</td>
<td>Sporogenous hyphae not delimited by a septum</td>
</tr>
<tr>
<td>Sporiferous saccule well developed prior to spore primodium formation</td>
<td>Sporiferous saccule and spore primodia developing simultaneously</td>
</tr>
<tr>
<td>Cytoplasm within the sporiferous saccule differentiated into regions of high and low density</td>
<td>Cytoplasm within the sporiferous saccule not differentiated into regions of different density</td>
</tr>
<tr>
<td>Sporiferous saccule sometimes germinating directly producing germination hyphae</td>
<td>Germination or sporiferous saccule not observed</td>
</tr>
<tr>
<td>Spores developed laterally</td>
<td>Spores developed laterally</td>
</tr>
<tr>
<td>Second septum delimiting the empty sporiferous saccule</td>
<td>No delimitation of empty sporiferous saccule</td>
</tr>
<tr>
<td>Sporiferous saccule shriveling at spore maturity</td>
<td>Sporiferous saccule shriveling at spore maturity</td>
</tr>
</tbody>
</table>

gw2 are not permanently fixed in place (unless spores have been stored for more than 60 days in formalin or some other preservative) and can disperse upon crushing of the spore and disappear (http://invam.caf.wvu.edu). They also appear to dissolve or lose refractivity in PVLG-based mountants after 30-90 days (varying with condition of spores and fungal species). The third wall layer (L3) is analogous to L3 found in other species with ornamented spore walls, such as *A. rehmi* and *A. tuberculata*, and species with a smooth spore wall, such as *A. mellea* (Stürmer & Morton 1999). In some other species (e.g. *A. koskei* and *A. laevis*), this layer is truly distinct and has sublayers much like L2 (Stürmer & Morton 1999, Blaszkowski 1995). The differences between *A. scrobiculata* and *A. rehmi* (Table 2) clearly indicate that differences in sporogenesis can occur among different species of *Acaulospora*.

Acknowledgement

T.M. and V.R. thank the Council of Scientific and Industrial Research, New Delhi, for financial support.

References


488


Received 31 August 2004, accepted in revised form 21 February 2005.